

### Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

#### Listing of Claims:

1. (Currently Amended) A method for detecting the methylation status of a nucleotide at a predetermined position in a nucleic acid molecule comprising:

- (a) treating a sample comprising said nucleic acid molecule in an aqueous solution with an agent suitable for the conversion of said nucleotide if present in

- (i) methylated form; or

- (ii) non-methylated form

to pair with a nucleotide normally not pairing with said nucleotide prior to

conversion;

- (b) amplifying said nucleic acid molecule treated with said agent via at least one amplification primer, wherein said at least one amplification primer is detectably labeled with a detectable label that forms an anchor for removal of single stranded amplified nucleic acid molecules to generate ~~an~~ a single stranded amplified nucleic acid molecule;

- (c) real-time sequencing said single stranded amplified nucleic acid molecule; and

- (d) detecting whether said nucleotide is methylated or not methylated at said predetermined position in the sample.

2. (Original) The method of claim 1 wherein said sample is derived from a tissue, a body fluid or stool.

3. (Original) The method of claim 2 wherein said tissue is a tumor tissue, neurodegenerative tissue or a tissue affected with another neurological disorder.

4. (Previously Presented) The method of claim 1 wherein said nucleic acid molecule is a DNA molecule or an RNA molecule.

5. (Previously Presented) The method of claim 1 wherein in (b) the nucleic acid molecule is amplified via LCR or PCR.

6. (Cancelled)

7. (Currently Amended) The method of claim ~~6~~ 1 wherein said amplification primer is labeled with (a) biotin, (b) avidin, (c) streptavidin or (d) a derivative of (a), (b) or (c) or a magnetic bead.

8. (Previously Presented) The method of claim 1 wherein said methylated nucleotide is an adenine, guanine or a cytosine.

9. (Previously Presented) The method of claim 1

wherein said real-time sequencing comprises:

(a) hybridization of a sequencing primer to said amplified nucleic acid molecule in single-stranded form;

(b) addition of a DNA polymerase, a ATP sulfurylase, a luciferase, an apyrase, adenosine-phosphosulfate (APS) and luciferin;

(c) sequential addition of all four different dNTPs;

(d) detection of a luminescent signal wherein an intensity of the luminescent signal is correlated with the incorporation of a specific nucleotide at a specific position in the nucleic acid molecule and wherein the intensity of said signal is indicative of the methylation status of said nucleotide at said predetermined position.

10. (Currently Amended) The method of claim 1, further comprising quantifying calculating a frequency of the methylated nucleotides from results of said real-time sequencing.

11. (Previously Presented) The method of claim 1 wherein said agent suitable for the conversion of said nucleotide to pair with nucleotide normally not pairing with said nucleotide is a bisulfite, preferably sodium bisulfite.

12. (Currently Amended) A method for the diagnosis of a pathological condition or the predisposition for a pathological condition comprising detection of the methylation status of a nucleotide at a predetermined position in a nucleic acid molecule comprising:

(a) treating a sample comprising said nucleic acid molecule in an aqueous solution with an agent suitable for the conversion of said nucleotide if present in

- (i) methylated form; or
- (ii) non-methylated form

to pair with a nucleotide normally not pairing with a said nucleotide prior to conversion;

(b) amplifying said nucleic acid molecule treated with said agent via at least one amplification primer, wherein said at least one amplification primer is detectably labeled with a detectable label that forms an anchor for removal of single stranded amplified nucleic acid molecules to generate ~~an~~ a single stranded amplified nucleic acid molecule;

(c) real-time sequencing said single stranded amplified nucleic acid molecule; and

(d) detecting whether said nucleotide is methylated or not methylated at said predetermined position in the sample ~~wherein a methylated or a not methylated nucleotide is indicative of a~~ to diagnose said pathological condition or the predisposition for said pathological condition.

13. (Previously Presented) The method of claim 12 wherein said pathological condition is cancer, a neurodegenerative disease or another neurological disorder.

14. (Original) The method of claim 13 wherein said cancer is a primary tumor, a metastasis or a residual tumor.

15. (Original) The method of claim 14 wherein said primary tumor is a

glioma.

16. (Previously Presented) The method of claim 15 wherein said glioma is an astrocytoma, oligodendroglioma, an oligoastrocytoma, a glioblastoma, or a pilocytic astrocytoma.

17. (Currently Amended) The method of claim ~~43~~ 38 wherein said neurodegenerative disease is Alzheimer's disease, Parkinson disease, Huntington disease, or Rett-Syndrome.

18. (Currently Amended) The method of claim ~~43~~ 38 wherein said neurological disorder is Prader-Willi-Syndrom, Angelman-Syndrome, Fragile-X-Syndrome, or ATR-X-Syndrome.

19. (Previously Presented) The method of claim 12 wherein said nucleic acid molecule is a DNA molecule or an RNA molecule.

20. (Previously Presented) The method of claim 12 wherein in (b) the nucleic acid molecule is amplified via LCR or PCR.

21. (Cancelled)

22. (Currently Amended) The method of claim ~~24~~ 1 wherein said amplification primer is labeled with (a) biotin, (b) avidin, (c) streptavidin or (d) a derivative of (a), (b or (c) or a magnetic bead.

23. (Previously Presented) The method of claim 12

wherein said methylated nucleotide is an adenine, guanine or a cytosine.

24. (Previously Presented) The method of claim 12

wherein said real-time sequencing comprises:

(a) hybridization of a sequencing primer to said amplified nucleic acid molecule in single-stranded form;

(b) addition of a DNA polymerase, a ATP sulfurylase a luciferase, an Apyrase, adenosine-phosphosulfate (APS) and luciferin;

(c) sequential addition of all four different dNTP's

(d) detection of a luminescent signal wherein the intensity of the luminescent signal is correlated with the incorporation of a specific nucleotide at a specific position in the nucleic acid molecule and wherein the intensity of said signal is indicative of the methylation status of said nucleotide at said predetermined position.

25. (Currently Amended) The method of claim 12 further comprising

quantifying calculating a frequency of the methylated nucleotides from results of said real-time sequencing.

26. (Previously Presented) The method of claim 12

wherein said agent suitable for the conversion of said nucleotide to pair with a nucleotide normally not pairing with said nucleotide is a bisulfite, preferably sodium bisulfite.

27. (Previously Presented) The method of claim 1

wherein said method is a high-throughput method.

28. (Previously Presented) The method of claim 12 wherein said sample is derived from tissue, a body fluid or stool.

29. (Previously Presented) The method of claim 28 wherein said body fluid is blood, serum or urine.

30. (Previously Presented) The method of claim 1 wherein said nucleotide is a cytosine and is part of one of the following sequences: CpG, CpNpG or CpNpN.

31. (Previously Presented) The method of claim 1, wherein the methylation status of more than one predetermined nucleotide is detected and a number of samples are analyzed at the same time.

32. (Currently Amended) A method for generating new nucleotide pairing partners upon amplification of at least one nucleic acid molecule for the detection of the methylation status of nucleotides of said nucleic acid molecule, said method comprising:

- a. providing said at least one nucleic acid molecule;
- b. treating said nucleic acid molecule with an agent suitable for conversion of a nucleotide if present in methylated form or non-methylated form to pair with nucleotide pairing partners normally not pairing with said nucleotide prior to conversion;
- c. amplifying said nucleic acid molecule via at least one amplification primer, wherein said at least one amplification primer is detectably labeled with a detectable label that forms an anchor for removal of single stranded amplified nucleic acid molecules to produce an amplification product generate a single stranded amplified nucleic acid molecule comprising

said new nucleotide pairing partners normally not pairing with said nucleotide prior to conversion and;

- d. real-time sequencing said ~~amplification product~~ single stranded amplified nucleic acid molecule;
- e. determining the amount of said nucleotide pairing with said new nucleotide pairing partners ~~in said amplification product~~ to detect the methylation status of nucleotides of said nucleic acid molecule.

33. (Previously Presented) The method of claim 1, wherein the methylation status of more than one predetermined nucleotide is determined.

34. (Currently Amended) The method of claim 10, further comprising detecting an allele frequency, wherein an allele frequency of 5% can be detected.

35. (New) The method of claim 1, wherein said primer does not comprise CpG.

36. (New) The method of claim 1, wherein all nucleotides formerly methylated or not methylated in said nucleic acid molecule are detected.

37. (New) The method of claim 8, wherein said methylated nucleotide is an adenine or guanine.

38. (New) The method of claim 12, wherein said pathological condition a neurodegenerative disease or another neurological disorder.